# THE INFLUENCE OF SOME SPRAY MATERIALS ON THE CHLOROPHYLL CONTENT OF APPLE LEAVES

by

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# INTRODUCTION

The purpose of this study was to determine whether the chlorophyll content of apple leaves was affected quantitatively when the following sprays, DDT (Dichloro diphenyl trichloroethane), Fermate (Ferric dimethyldithiocarbamate) -lead arsenate, wettable sulphur-lead arsenate and zinc sulphate-lead arsenate were applied. Zinc sulphate-lead arsenate is the approved insecticide for the control of the codling moth in Kansas. DDT is showing considerable promise as an insecticide also in the control of the codling moth, but requires further tests in the control of fruit insects. Fermate, an organic compound, bids fair to gain a place in the spray schedule as it shows considerable promise for the control of such diseases as apple scab and cedar rust. Wettable sulphur is a standard fungicide for the control of apple scab and other fungous diseases. Sulphur and lead arsenate and Fermate-lead arsenate are applied together commercially because of convenience as combination sprays.

The role of chlorophyll in photosynthesis has not been agreed upon by investigators but is considered by all to play a vital part in the process. Sachs (1882) stated that the amount of chlorophyll was a limiting factor of the rate of photosynthesis. Further study of factors which might alter the chlorophyll content seems highly desirable.

## REVIEW OF LITERATURE

The first chemical investigation of chlorophyll was made by Berzelius a little over a century ago (1838), according to Rabinowitch (1945). He lists, as an important step forward in the long gradual unveiling of the structure of this compound, the realization of the similarity between chlorophyll and hemin, the red blood pigment, first suspected by Verdein in 1851 and later confirmed by Hoppe-Seyler (1879, 1880, 1881) who transformed chlorophyll into a red "porphyrin" similar to those obtainable from hemin. Sachs (1882) recognized that chlorophyll was one of the factors affecting assimilation, which was later referred to as photosynthesis, although knowledge of the chloroplast was uncertain. It remained for Willstatter and his co-workers during the early part of the twentieth century to contribute the first comprehensive study of the subject. They succeeded in isolating the pigments in a pure state, and also succeeded in determining their chemical nature.

Further investigations of the properties of chlorophyll and methods of extraction and determination have been conducted by Dastur and Buhariwalla (1926), Willstatter and Stoll (1928), Sprague and Shive (1929), Harriman (1930), Peterson (1930), Fleischer (1934), Zscheile (1934), Kuhn (1935), Loomis and Shull (1937), Johnston and Weintraub (1939), Mackinney (1940), Zscheile and Comar (1941), Aronoff and Mackinney (1943), Benne, Rose and Comar (1944) and Compton and Boynton (1945).

Willstatter and Stoll (1928) reported that the chloroplasts contain four pigments: the green pigments, chlorophyll component a, chlorophyll component b, and two yellow pigments, carotene and xanthophyll. The term "chlorophyll" as it is now commonly used refers to the two green pigments taken collectively and freed from all the others that are associated with it in the chloroplasts according to Miller (1938).

Carbon, hydrogen, oxygen, nitrogen and magnesium are the elements comprising chlorophyll as found by Willstatter and Stoll (1928). Miller (1938) reports that chlorophyll always contains 2.7 per cent magnesium, which is the only metal in the ash.

According to the work of Willstatter and Stoll (1928), the evidence seems to indicate that chlorophyll is present in the chloroplasts in a colloidal mixture.

Thomas (1935) states that the most probable explanation of the fact that photosynthesis only occurs in green leaves is that the absorption of light-energy for the conversion of carbon dioxide and water into carbohydrates is dependent on the presence of the chloroplast pigments. Maximov (1930) states that chlorophyll absorbs light, not as a whole, but selectively. Certain portions of the spectrum are absorbed by it while others are transmitted. Miller (1938) reports that the spectrum of chlorophyll shows that there are five absorption bands. The most distinct band includes the red rays of 651 to 680 millimicrons approximately on both sides of the Fraunhofer line C. It is in this band, according to Maximov, that the most intense absorption takes place.

Besides the selective absorption of light-energy, chlorophyll posesses another important optical property, fluorescence. Miller (1938) states the action generally is to increase the wave lengths, but in some few cases it is the reverse. Usually the yellow, green, and blue rays undergo the change. In the alcoholic solution of chlorophyll, the much shorter wave lengths of green and blue are emitted as the longer wave lengths of the red. Thus, an alcoholic solution of chlorophyll a is blue-green by transmitted light and blood-red by reflected light. This is due to an alteration in the wave lengths of radiant energy brought about by the action of the molecules of the substance.

Miller (1938) states that the formation of chlorophyll is a physiological process that occurs only in living cells and under conditions favorable to life. The substances or substance from which chlorophyll arises have never been isolated, and their existence is only inferred. Palladin (1922) considers that a pigment called "chlorophyllage" is formed independently of light in the chloroplasts, and that it is rapidly transformed into chlorophyll under the influence of light. According to Eyster (1928) the absorption spectrum of the pigments obtained from etiolated seedlings has an absorption band between 640 and 620 millimicrons which is due to a red fluorescing pigment that has been termed "protochlorophyll". This is a pigment that develops without the aid of light and changes photo-chemically into chlorophyll upon exposure to light.

Light is one of the requisites for the formation of chlorophyll. Most plants grown in the darkness posess yellow leaves

and stems. Some plants or plant parts such as the seedlings of some conifers, the fronds of young ferns and certain unicellular algae are exceptions to this since they become green in darkness, Miller (1938). According to Palladin (1922), the conifer seedlings, however, form much less chlorophyll in darkness than in light. Miller (1938) states that light of medium intensity is the most favorable for chlorophyll formation. It is considered that in more intense light the formation and decomposition of chlorophyll occur simultaneously, so, as a net result, the greening is less pronounced than it is in diffused light.

Sayre (1928) grew plants under colored glass plates and studied the effect of different wave lengths of radiant energy on the formation of chlorophyll in the seedlings of corn, wheat, oats, barley, sunflowers and radish. The wave lengths longer than 680 millimicrons are not effective in the formation of chlorophyll, but all other regions of the remaining visible and ultraviolet spectrum to 300 millimicrons are effective provided the energy value is sufficient. For equal energy values, the red rays are more effective than the green, and the green more than the blue. Ulvin (1934) found that the leaves of radishes grown in continuous light contained more chlorophyll than those grown in a ten hour daily light period.

Miller (1938) states that the rate of production of chlorophyll depends upon temperature. Greening occurs most quickly in etiolated plants between 18 to 30 degrees Centigrade. He reports that Wiesner found within that range of temperature that greening occurs in about one and six tenths hours, while at ten

degrees Centigrade, three and five tenths hours were required. Lubinenko and Hubbenet noted, according to Miller (1938), that the greening of etiolated wheat seedlings takes place within definite limits of temperature, beginning between two to four degrees Centigrade, attains a maximum between 26 to 30 degrees Centigrade and ceases at or near 48 degrees Centigrade.

Miller (1938) noted that the elements iron, potassium, phosphorus, calcium and magnesium all influence the development of chlorophyll. Schertz (1929) associated the mottling of Coleus plants with a deficiency in nitrates. Deuber (1926) noted that in the soybean the chloroplast pigments increased with the increasing concentration of iron and sulphur in the cultural solutions. Deuber, however, could not establish an exact proportional relation between the concentration or amount of any of the elements studied and the amount of pigment formed. Schertz (1929) observed that phosphorus, potassium and nitrogen may each be correlated with an effect on the formation of the chloroplast pigments. Ulvin (1934) reported that with sugar cane more chlorophyll was produced by plants receiving nitrogen in the form of nitrate than by those receiving this element in the form of ammonia.

Palladin (1922) has reported that carbohydrates in the leaves are essential to the formation of chlorophyll.

Miller (1938) reports Henrici as having found, in South Africa, that there was a pronounced difference between the amount of chlorophyll present in the morning and in the afternoon. Following a heavy rain there was an increase in the chlorophyll content, while drought caused a decrease.

Lubimenko, as reported by Miller (1938), found that shade plants could accomplish the same amount of photosynthesis with a lower illumination than the sun plants and that the chlorophyll content of the former was higher than that of the latter. The sun plants with the low chlorophyll content showed the maximum rate of photosynthesis at the highest light intensities, while the shade plants showed a decreased rate at the same intensities. He considered that leaves with high chlorophyll content have a high absorption coefficient and that the optimum temperature and light intensity for photosynthesis decrease with chlorophyll content. Thus, in the course of the development a plant can regulate the amount of light it absorbs by changes in its chlorophyll content. According to Miller, Henrici made observations in agreement with those of Lubimenko, since she noted that lowland plants may have as much as two and three tenths times more chlorophyll than the alpine plants of the same species and in strong light the rate of photosynthesis in the alpine plants was higher than in the lowland plants.

Spoehr (1926) reports Willstatter and Stoll as having observed that the rate of photosynthesis increased with the chlorophyll content, but they were not able to establish any relation between these two factors. Fleischer (1934) reports essentially the same findings. Spoehr believed that chlorophyll is but one part of a complex mechanism to the successful function of which other parts are essential. He believed that other factors such as protoplasmic factors and enzymes also affect the rate of photosynthesis.

The most important factors affecting the quantity of pigments are rainfall, soil moisture, nutrient elements in the soil, light intensity, temperature and relative humidity, according to Schertz (1929).

Demerec, as reported by Miller (1938), states that chlorophyll shows more peculiarities in its inheritances than any other known plant characteristic. In maize there are approximately 100 genetically different characters for chlorophyll.

Emerson (1929) lists two sets of factors, external and internal that are recognized as affecting the rate of photosynthesis in green plants. Light, temperature and the supply of carbon dioxide are among the external factors. The internal factors are not well known or understood, but chlorophyll is one of them. Emerson also found when studying the rate of photosynthesis with various light intensities and varying chlorophyll concentrations that photosynthesis reached its maximum rate at about the same light intensities over the whole range of chlorophyll concentration used. The same relationship existed with temperature. The results indicate that photosynthesis may involve an autocatalytic reaction and that chlorophyll plays some part in the process in addition to its role in light absorption.

Pickett (1937) found that leaves of the York apple variety had a slightly greater amount of chlorophyll per unit area of leaf area than those of the Wealthy variety. Due to the greater amount of internally exposed surface in the mesophyll cells of the Wealthy leaves, he concluded that the chlorophyll of the leaves of the variety could enter into photosynthetic activity more effectively than the chlorophyll of the York leaves.

Pickett and Kenworthy (1940) stated that the amount of chlorophyll is not as significant in the process of photosynthesis as is the internal structure of the leaves.

There is little evidence in the literature of the influence of spray materials upon the chlorophyll content of plant tissue. According to Hyre (1939), liquid lime-sulphur and arsenate of lead sprays will decrease the rate of photosynthesis even when the leaves appear uninjured. Ruth (1922) found, in the case of the common bean, Phaseolus vulgaris, that the chlorophyll content of a given unit of leaf area of the primordial leaves sprayed with a Bordeaux mixture was slightly greater than the chlorophyll content of the same unit of area of the unsprayed primordial leaves. He observed that the chlorophyll content per unit area of the primordial leaves decreased as the leaves developed after the shedding of the cotyledons. The leaves of the sprayed plants did not equal in size those which were not sprayed. Ginsburg (1929) found that Wealthy and Gravenstein apple leaves sprayed with oil had a greater amount of chlorophyll than the unsprayed leaves of the same varieties. His explanation of the greater chlorophyll content in the sprayed leaves of the two varieties is as follows: (1) oil sprays may stimulate directly the chloroplast formation in the epidermal cells of the leaf, (2) greater reduction of leaf hoppers on the sprayed leaves compared with the unsprayed leaves, and (3) the spray may reduce the light intensity. According to Palladin (1922), chlorophyll accumulates faster in weak mid-light than in strong light which causes decomposition of chlorophyll.

Saunders (1941) found that the chlorophyll content of greenhouse grown York and Wealthy apple leaves was reduced by spraying with arsenate of lead and liquid lime-sulphur. He advanced the idea that this reduction in chlorophyll as compared to the unsprayed leaves might be due to reduced light intensity caused by the spray residue on the leaf. He found that in field grown trees the chlorophyll content was not reduced by spraying, but that a great variation occurred between the unsprayed and sprayed leaves at the various dates. Saunders believed the variations were induced by environmental factors.

## MATERIALS AND METHODS

Thirty, two-year old Jonathan and twenty, two-year old Winesap trees were planted in the field in March 1946. The two varieties were segregated and planted with each variety occupying three rows, and the trees spaced six feet apart on the square. The spray treatments were selected at random for each tree within a variety.

Five treatments were compared. They included (1) two pounds of 25 per cent wettable DDT to 50 gallons of water, (2) one pound of Fermate and one and one-half pounds of lead arsenate to 50 gallons of water, (3) four pounds of wettable sulphur and one and one-half pounds of lead arsenate to 50 gallons of water, (4) four ounces of zinc sulphate and two pounds of lead arsenate to 50 gallons of water and (5) the checks to which no sprays were applied.

A canvas hood was placed over each tree during spraying to prevent any of the spray from getting on trees selected for different treatments. Eight applications of each spray were made. The spray dates were: May 20 and 27, June 3, 10, 17 and 24 and July 1 and 8.

Sampling and Extraction of Chlorophyll

Chlorophyll determinations were made on the following nine dates: May 31, June 7, 13, 20 and 27, July 5, 12 and 19 and August 9, 1946.

To obtain relatively comparable values for chlorophyll over a period of several weeks, a dated tag was placed on the youngest leaf on each shoot at two-week intervals and leaves were selected from the middle portion of the shoot.

Five leaves were selected each time from the middle portion of the shoots taking care to select shoots in the same relative position on the tree, thus, helping to insure representative sampling.

Discs with an area of one square centimeter were cut from fresh leaves and placed immediately in the solvent. When taking the samples the midribs of leaves were avcided. All discs were taken from the same relative position on the leaves beginning at the head portion of the leaf and taking an equal number of discs from each side of the midrib.

The method of chlorophyll extraction and determination used was the one suggested by Compton and Boynton (1945) with modifications. The method is based on the fact that the strong

absorption band in the red end of the spectrum for chlorophyll is not overlapped by those of the carotenoid pigments.

Thirty discs, one square centimeter in area, were cut from fresh leaves and placed immediately in 30 milliliters of methanol to which a small amount of cupric nitrate was added. The leaf tissue was left to stand in the solvent for a minimum of 24 hours and then mixed in a Waring blendor for three to four minutes. The solution was then filtered through a Buchner funnel fitted with Whatman 44 filter paper and transferred into a 100 milliliter volumetric flask and made up to volume by washing the pulp with several portions of the solvent. The extract was read in a Klett-Summerson photoelectric colorimeter using a Corning No. 2408 light filter which transmitted above 610 millimicrons.

A sampling error of two and six hundreths per cent was calculated for the method of chlorophyll extraction and determination.

## Calibration of Colorimeter

The colorimeter was calibrated by the method described by Benne, Rose and Comar (1944).

One hundred milliliters of the extract were prepared as described above, washed in a separation funnel with di-ethyl ether and water until all the methanol was removed (5-10 washings). The ether solution was filtered through anhydrous $Na_2SO_4$, and then transferred to a 100 milliliter volumetric flask and made up to volume with more di-ethyl ether. Aliquots of this solution were removed and read together with the original

solution on the Beckman quartz spectrophotometer and a calibration curve established.

The reading total chlorophyll calculated as milligrams per liter was expressed as milligrams per square meter of leaf area.

The dates of spray application and chlorophyll determination and a list of the spray materials used are presented in Table 1.

Table 1. Schedule of spray applications and chlorophyll determinations. Field grown trees, 1946.

| Spray application dates | Chlorophyll determination dates |
|---|---|
| May 20 | May 31 |
| May 27 | June 7 |
| June 3 | June 13 |
| June 10 | June 20 |
| June 17 | June 27 |
| June 24 | July 5 |
| July 1 | July 12 |
| July 8 | July 19 |
| | August 9 |

Spray Materials Used

DDT - two pounds of 25 per cent wettable powder to 50 gallons of water.

Fermate - one pound and lead arsenate one and one-half pounds to 50 gallons of water.

Wettable sulphur - four pounds and lead arsenate one and one-half pounds to 50 gallons of water.

Zinc sulphate - four ounces and lead arsenate two pounds to 50 gallons of water.

# PRESENTATION OF DATA

The data presented were subjected to an analysis of variance as described by Paterson (1939). As the chlorophyll content was highly variable it was apparent that the results of the observations must be treated statistically before formulating conclusions.

The average chlorophyll content of Jonathan apple leaves for each spray treatment at each date is given in Table 2. The corresponding analysis of variance is found in Table 3.

Table 2. Average chlorophyll content of Jonathan apple leaves for each spray treatment at each date expressed in milligrams per square meter of leaf area, 1946.

| Date | DDT | Fermate-lead arsenate | Sulphur-lead arsenate | Zinc sulphate-lead arsenate | Checks | Average |
|---|---|---|---|---|---|---|
| May 31 | 306.1 | 276.1 | 304.8 | 337.6 | 287.1 | 302.3 |
| June 7 | 257.3 | 258.1 | 270.6 | 290.8 | 258.1 | 266.9 |
| June 13 | 309.0 | 312.5 | 304.8 | 307.5 | 309.8 | 308.7 |
| June 20 | 256.3 | 245.6 | 235.8 | 259.3 | 253.1 | 250.0 |
| June 27 | 325.1 | 306.1 | 299.6 | 328.5 | 291.6 | 310.1 |
| July 5 | 335.1 | 316.8 | 302.8 | 305.3 | 315.1 | 315.0 |
| July 12 | 324.5 | 338.0 | 342.3 | 334.0 | 330.1 | 333.7 |
| July 19 | 347.6 | 344.0 | 347.0 | 298.1 | 355.1 | 338.3 |
| August 9 | 369.0 | 368.3 | 392.3 | 371.3 | 390.8 | 378.3 |
| Average | 314.4 | 307.2 | 311.1 | 314.7 | 310.0 | |

Table 3. Analysis of variance of the chlorophyll content of the Jonathan apple leaves from two-year old field grown trees, 1946.

| Factor | Degrees of freedom | Sum of squares | Variance |
|---|---|---|---|
| Total | 269 | 705,581.8 | |
| Between dates | 8 | 343,909.8 | 42,988.70** |
| Between spray treatments | 4 | 919.4 | 229.85 |
| Interaction | 32 | 46,720.9 | 1,460.02 |
| Error | 225 | 314,317.0 | 1,396.96 |

** Variance which is significant at the one per cent level as shown by the F test.

The variation in the amount of chlorophyll between dates was so great that the value of F was 30.77 whereas a value of but 2.53 is significant at the one per cent level. This indicates that the chlorophyll content can be highly variable at different periods during the growth of Jonathan apple leaves.

The variation in chlorophyll content between spray treatments was very small compared with the variation due to error. In fact, an F value of at least 2.41 was necessary for significance at the five per cent level but a value of only 0.164 occurred indicating that the observed difference between the spray treatments used in this study were not significant.

The average chlorophyll content of Winesap apple leaves for each spray treatment at each date is given in Table 4 and the corresponding analysis of variance is found in Table 5.

Table 4. Average chlorophyll content of Winesap apple leaves for each spray treatment at each date as expressed in milligrams per square meter of leaf area, 1946.

| Date | DDT | Fermate-lead arsenate | Sulphur-lead arsenate | Zinc sulphate-lead arsenate | Checks | Average |
|---|---|---|---|---|---|---|
| May 31 | 265.7 | 288.5 | 268.7 | 247.2 | 286.7 | 271.4 |
| June 7 | 279.5 | 282.5 | 291.5 | 260.2 | 311.5 | 285.0 |
| June 13 | 343.0 | 372.0 | 288.0 | 343.5 | 392.2 | 347.7 |
| June 20 | 269.7 | 278.7 | 230.2 | 261.0 | 268.0 | 261.5 |
| June 27 | 347.7 | 310.5 | 324.7 | 309.7 | 318.0 | 322.1 |
| July 5 | 373.2 | 310.5 | 336.0 | 356.5 | 373.0 | 349.8 |
| July 12 | 412.5 | 382.5 | 377.0 | 360.7 | 424.5 | 391.4 |
| July 19 | 373.0 | 304.0 | 363.5 | 375.5 | 346.2 | 352.4 |
| August 9 | 392.7 | 457.5 | 456.2 | 431.5 | 462.0 | 439.9 |
| Average | 339.7 | 331.9 | 326.2 | 327.3 | 353.6 | |

Table 5. Analysis of variance of the chlorophyll content of the Winesap apple leaves from two-year old field grown trees, 1946.

| Factor | Degrees of freedom | Sum of squares | Variance |
|---|---|---|---|
| Total | 179 | 868,980.73 | |
| Between dates | 8 | 539,885.28 | 67,435.66** |
| Between spray treatments | 4 | 18,371.81 | 4,592.95* |
| Interaction | 32 | 75,779.14 | 2,368.09 |
| Error | 135 | 234,944.50 | 1,740.32 |

** Variance which is significant at the one per cent level as shown by the F test.
* Variance which is significant at the five per cent level as shown by the F test.

The analysis of variance showed that the variation in chlorophyll content between dates was significantly greater than the variation due to error. To show significance at the one per cent level, an F value of 2.82 was necessary, whereas, an F value of 38.77 occurred. This indicates that the amount of chlorophyll in Winesap leaves can be highly variable during the growing season, as was previously shown to be true for Jonathan apple leaves.

The variation in the chlorophyll content due to spraying was significantly greater than the variation due to error. An F value of 2.46 at the five per cent level was necessary for significance, while a value of 2.63 was obtained. In order to determine which differences among the sprays were important,

the following individual comparisons were made of their effect on the chlorophyll content of the leaves.

| | | |
|---|---|---|
| Fermate-lead arsenate | vs | sulphur-lead arsenate and zinc sulphate-lead arsenate |
| Sulphur-lead arsenate | vs | zinc sulphate-lead arsenate |
| DDT | vs | arsenic sprays |
| Untreated checks | vs | actual sprays |

These tests indicated that spraying may reduce the chlorophyll content of the leaves; but all the observed differences among the different types of sprays were found to be within the bounds of sampling variations.

While the interaction is not significant there is considerable shifting around of the chlorophyll content of the leaves treated with the different sprays at the various dates as shown in Table 4. The between spray treatment variance is not significantly greater than that for the interaction variance.

Figure 1 graphically presents the relationship of the average chlorophyll content of the sprayed to the unsprayed leaves of the Winesap variety at the different dates.

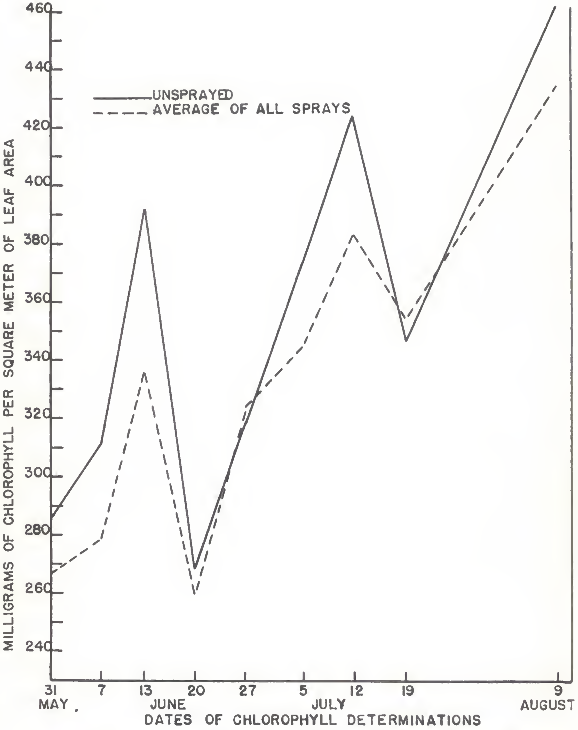


FIGURE I. CHLOROPHYLL CONTENT IN MILLIGRAMS PER SQUARE METER OF LEAF AREA OF SPRAYED AND UNSPRAYED WINESAP APPLE LEAVES. 1946.

The data on chlorophyll content of sprayed and unsprayed Jonathan and Winesap leaves pooled are presented in Table 6 with the corresponding analysis of variance which is found in Table 7.

Table 6. Chlorophyll content of the Jonathan and Winesap apple leaves from two-year old field grown trees, 1946.

| Dates of determination | Tree number | | Variety | Chlorophyll per sq. meter leaf area in mg. | | | | |
|---|---|---|---|---|---|---|---|---|
| | Unsprayed | Sprayed | | Unsprayed | Sprayed | Average of all readings | Average of unsprayed | Average of sprayed |
| May 31 | 6 | 24 | Jonathan | 287.1 | 281.1 | | | |
| | 4 | 16 | Winesap | 286.7 | 267.5 | 280.6 | 286.9 | 274.3 |
| June 7 | 6 | 24 | Jonathan | 258.1 | 269.2 | | | |
| | 4 | 16 | Winesap | 311.5 | 278.4 | 279.3 | 284.8 | 273.8 |
| June 13 | 6 | 24 | Jonathan | 309.8 | 308.4 | | | |
| | 4 | 16 | Winesap | 392.2 | 336.6 | 336.7 | 351.0 | 322.5 |
| June 20 | 6 | 24 | Jonathan | 253.1 | 249.2 | | | |
| | 4 | 16 | Winesap | 268.0 | 259.9 | 257.5 | 260.5 | 254.5 |
| June 27 | 6 | 24 | Jonathan | 291.6 | 314.8 | | | |
| | 4 | 16 | Winesap | 318.0 | 323.1 | 311.8 | 304.8 | 318.9 |
| July 5 | 6 | 24 | Jonathan | 315.1 | 315.0 | | | |
| | 4 | 16 | Winesap | 373.0 | 344.0 | 336.7 | 344.0 | 329.5 |
| July 12 | 6 | 24 | Jonathan | 330.1 | 334.7 | | | |
| | 4 | 16 | Winesap | 424.5 | 383.1 | 368.1 | 377.3 | 358.9 |
| July 19 | 6 | 24 | Jonathan | 355.1 | 334.1 | | | |
| | 4 | 16 | Winesap | 346.2 | 354.0 | 347.3 | 350.6 | 344.0 |
| August 9 | 6 | 24 | Jonathan | 390.8 | 375.2 | | | |
| | 4 | 16 | Winesap | 462.0 | 434.4 | 415.6 | 426.4 | 404.8 |
| Average | | | | 331.7 | 320.1 | | | |
| Average of Jonathan | | | | 310.0 | 309.0 | 309.5 | | |
| Average of Winesap | | | | 353.5 | 331.2 | 342.3 | | |

Table 7. Analysis of variance of the average chlorophyll content of the Jonathan and Winesap apple leaves pooled from field grown two-year old trees, 1946.

| Factor | Degrees of freedom | Sum of squares | Variance |
|---|---|---|---|
| Total | 89 | 237,425.75 | |
| Between varieties | 1 | 11,913.77 | 11,913.77** |
| Between dates | 8 | 176,571.05 | 22,071.38** |
| Between spray treatments | 4 | 2,950.65 | 737.66 |
| Interactions: Varieties x dates | 8 | 17,447.71 | 2,180.96** |
| Varieties x treatment | 4 | 3,248.93 | 812.23 |
| Dates x treatment | 32 | 11,712.53 | 366.01 |
| Error | 32 | 13,381.11 | 424.09 |

** Variances which are highly significant at the one per cent level as shown by the F test.

A graphic comparison of the chlorophyll content of the Jonathan and Winesap leaves is given in Figure 2.

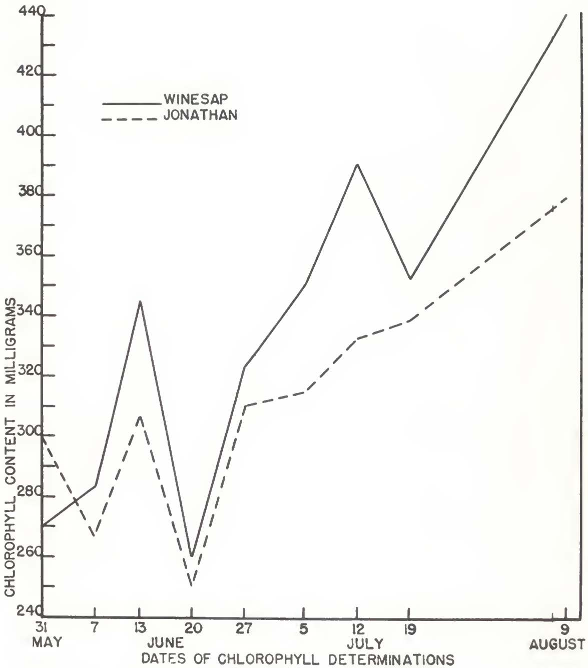


FIGURE 2. CHLOROPHYLL CONTENT OF SPRAYED AND UNSPRAYED JONATHAN AND WINESAP APPLE LEAVES FOR NINE DATES EXPRESSED IN MILLIGRAMS PER SQUARE METER OF LEAF AREA. 1946.

As shown in Table 6, the average chlorophyll content in the Winesap leaves was greater than that of the Jonathan leaves. The analysis of variance showed that this variation between the chlorophyll content of Winesap and Jonathan apple leaves was significantly greater than the variation due to error. An F value of 28.09 was obtained. To be highly significant at the one per cent level, an F value of 7.53 was necessary. This indicates that the chlorophyll content of Winesap apple leaves was considerably greater than that of Jonathan leaves. An average chlorophyll content for the Winesap and Jonathan apple leaves was 342.3 and 309.5 milligrams per square meter of leaf area, respectively.

The variation between the chlorophyll content of the nine dates was so great that an F value of 28.09 was obtained, whereas, an F value of at least 3.17 was necessary to be highly significant. This indicates that the average chlorophyll content of the two varieties for the nine dates was highly variable.

The variation in the chlorophyll content between the sprayed and unsprayed leaves of the two varieties was very small compared with the variation due to error. To be significant at the five per cent level, an F value of at least 2.67 must be obtained, while an F value of only 1.73 was found. This indicates that the amount of chlorophyll in the unsprayed leaves was not significantly greater than the amount in the sprayed leaves.

It was found that the variation in amount of chlorophyll between varieties and dates was so great that it was probably

not due to chance. An F value of 3.17 was necessary for significance, but a value of 5.14 occurred. This indicates that the varieties have reacted differently to the changing dates and the variation in chlorophyll content was considerable between the Jonathan and Winesap apple leaves at the nine dates. The average chlorophyll content for each variety at the nine dates is presented in Table 8.

Table 8. Average chlorophyll content of sprayed and unsprayed Jonathan and Winesap apple leaves for nine dates in milligrams per square meter of leaf area from two-year old field grown trees, 1946.

| Date | Winesap | Jonathan |
|---|---|---|
| May 31 | 271.40 | 302.40 |
| June 7 | 285.05 | 267.37 |
| June 13 | 347.75 | 308.73 |
| June 20 | 261.55 | 250.07 |
| June 27 | 322.15 | 310.23 |
| July 5 | 349.85 | 315.07 |
| July 12 | 391.45 | 353.80 |
| July 19 | 352.45 | 338.40 |
| August 9 | 439.93 | 378.34 |
| Average | 335.73 | 311.60 |

According to the analysis of variance, the interaction of varieties x sprays is not significant. To be significant at the five per cent level, an F value of at least 2.67 was

necessary, but a value of 1.91 occurred. This denotes that the varieties have reacted similarly to the various spray treatments.

The interaction dates x treatments was not significant. To be significant an F value of at least 1.74 was necessary, but a value of 0.86 occurred. The chlorophyll content of the leaves treated with the different sprays has, in general, reacted similarly at the various dates.

The relationship of the chlorophyll content of the Jonathan and Winesap leaves to the average daily mean temperature and precipitation for the period, May 31 to August 9, 1946, is shown in Figure 3.

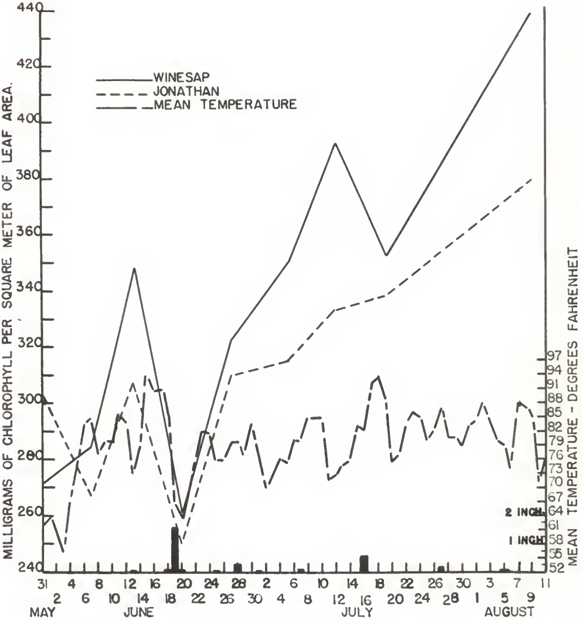


FIGURE 3. RELATIONSHIP OF CHLOROPHYLL CONTENT IN JONATHAN AND WINESAP APPLE LEAVES TO AVERAGE DAILY MEAN TEMPERATURE AND PRECIPITATION. 1946.

## DISCUSSION OF RESULTS

The amount of chlorophyll in the Jonathan apple leaves grown in the field varied greatly between dates. Due to the constantly changing environmental conditions, this might be expected. Bukatsch and Wendel, as reported by Rabinowitch (1945), have found large variations in the concentration of chlorophyll in a relatively short period of time in many species of plants. Ireland and Yeats (1933) found that the chlorophyll content of kafir leaves fluctuated widely throughout the growing season with the general trend being an increasing amount until maturity was reached, then an abrupt decrease was noted. Henrici (1926), working with grasses, found that the chlorophyll content varied during a twenty-four hour period and with the age of the leaf. Thus, the variation in chlorophyll content among dates in this study is in agreement with the investigations of other workers.

In the tests conducted with the Jonathan variety, the chlorophyll content of the leaves was not materially decreased by spraying. It is concluded that any variation in chlorophyll must be attributed to factors other than the different spray treatments, factors which were not measured in the experiment.

The amount of chlorophyll in the Winesap apple leaves was highly variable between dates. This was to be expected and is in agreement with the findings of the Jonathan leaves.

An analysis of variance showed that due to spraying the chlorophyll content produced variations which slightly exceeded the F value at the five per cent level of significance. As a

result of individual comparisons made of the sprays, it was indicated that spraying may reduce the chlorophyll content of the Winesap apple leaves. However, the test showed that there were no significant differences among the sprays.

There are two possible explanations to account for the decrease of chlorophyll content in the sprayed Winesap apple leaves. First, the genetical composition of the Winesap variety possibly accounts for the effect of the spray materials. All analyses showed a significant difference in the chlorophyll content of the Jonathan and Winesap varieties, with the average chlorophyll content of the Winesap leaves being considerably higher than that of the Jonathan leaves. Second, the Winesap leaves were apparently quite sensitive to external factors which might account for the decrease in chlorophyll content in the sprayed leaves. At the beginning of the experiment on May 31, the average chlorophyll content in the Jonathan apple leaves was higher than that of the Winesap variety. Possibly, as a result of favorable environmental conditions, the chlorophyll content increased rapidly in the Winesap leaves until on June 13 the average chlorophyll content was higher in the Winesap than in the Jonathan leaves. Following a five day period of relatively high mean temperatures, beginning on June 14, the average chlorophyll content dropped in both varieties though more sharply in the Winesap leaves. After a rain of 1.62 inches on June 19 and consequent lower temperatures, the chlorophyll content increased immediately in both varieties though more rapidly in the Winesap leaves. From July 16 to July 19

another period of relatively high temperatures occurred following which the chlorophyll content of the Winesap leaves dropped considerably while the drop in the chlorophyll content of the Jonathan leaves was not nearly so apparent. Thus, it is obvious that the Winesap variety was quite sensitive to environmental factors such as temperature, relative humidity and rainfall which significantly influenced the chlorophyll content. The chlorophyll content of the Winesap leaves, as a result of greater sensitiveity, might possibly be reduced by the application of the spray materials.

Schertz (1929) observed that the most important factors affecting the quantity of pigments are rainfall, soil moisture, nutrient elements in the soil, light intensity, temperature, and relative humidity. The period, May 31 to August 9, during which the investigation was carried on was characterized by hot dry weather. The total rainfall was 3.16 inches as opposed to the normal rainfall of 9.87 inches or a deficit of 6.71 inches. As a result of the five day period of high temperatures, beginning on June 14, there was a definite drop in chlorophyll content of all the leaves with the content dropping the lowest in the leaves sprayed with wettable sulphur-lead arsenate and the next lowest content in the zinc sulphate-lead arsenate sprayed leaves.

It was reported by Hyre (1939) that liquid lime-sulphur-lead arsenate will decrease the rate of photosynthesis even when the leaves appear uninjured. It was considered by Sachs (1882) that the amount of chlorophyll was a limiting factor of

the rate of photosynthesis. Saunders (1941) found that the chlorophyll content of greenhouse grown York and Wealthy apple leaves was reduced by spraying with lead arsenate and liquid lime-sulphur. He considered that the decrease in chlorophyll may have been brought about by the spray residue on the leaves which reduced the light intensity penetrating through the leaf to the point that chlorophyll formation was inhibited in the sprayed leaves.

As previously stated, the amount of chlorophyll in the leaves of both the Jonathan and the Winesap varieties varied greatly between dates.

According to the analysis of variance of the chlorophyll content of the sprayed and unsprayed leaves of the two varieties treated together, the variation was very small compared with the variation due to error. Neither were the interactions, variety x spray treatment or date x spray treatment, significant. The effect of spraying was thus not significant and could not be measured due to external factors, light, relative humidity, moisture supply and temperature which affected the chlorophyll content.

Any further study of this subject should include more comprehensive meteorological data as many of the environmental factors exert a considerable influence upon the chlorophyll content.

It would also be highly desirable to extend the study over an entire growing season to enable more accurate observations of seasonal behavior to be made.

Considerable work has been done recently in developing new methods for quantitative chlorophyll determinations, chief among these being the spectrophotometric method of analysis. This method seems to be a considerable improvement over previously known methods in that it is more accurate as well as time saving.

The work of Benne, Rose and Comar (1944) and others in developing a means of calibrating a photoelectric colorimeter for chlorophyll by use of a simple plant extract, a procedure that eliminates the necessity of isolating the chlorophyll thereby avoiding the possibility of pigment degradation, is an important contribution to the work.

## CONCLUSIONS

These data indicate the following:

1. The chlorophyll content of the Jonathan apple leaves was not reduced significantly by any of the four spray treatments applied.

2. The chlorophyll content of the Winesap apple leaves was slightly reduced by all the spray treatments but the observed differences between sprays were not significant.

3. The chlorophyll content of the Winesap leaves was more sensitive to external factors than that of the Jonathan variety. This greater sensitivity may account for the decrease in chlorophyll content due to the use of the spray materials.

4. The chlorophyll content of the Jonathan and Winesap leaves was variable between dates. The variation was highly significant.

5. The chlorophyll content of the Winesap variety was significantly greater than the amount found in the Jonathan variety growing under similar conditions.

6. A considerable seasonal variation in chlorophyll content in both varieties was noted with an increase of chlorophyll content observed with advancing age.

7. Prolonged periods of high temperatures materially reduced the chlorophyll content of both varieties.

8. The factors such as soil moisture, light intensity, soil nutrients, relative humidity and temperature probably were responsible for the large variation in chlorophyll content between dates of both sprayed and unsprayed leaves.

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